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ACUTE MYELOID LEUKEMIA

DNA damage response genes as biomarkers of therapeutic outcomes in acute myeloid leukemia patients

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TO THE EDITOR:
Current AML therapeutics induce DNA damage and/or modulate DNA damage response (DDR) directly or indirectly [1, 2]. Recent reports analyzed large datasets to predict the response of AML patients to the treatment [3–5]. The combinatorial mutational events (e.g., NRAS, TP53, TET2, IDH1 and/or NPM1) as well as single gene expression levels (e.g., PEAR1) were implicated as potential biomarkers of the clinical outcome. These genes however, except TP53, are not involved in DDR.

Here, we employed a list of 1800 DDR-related genes [6] to interrogate a cohort of 612 AML patients (612 specimens) from waves 1 to 4 of the training BEAT AML 2.0 dataset [3] to determine the correlations between DDR gene expression and therapeutic outcomes. Only 500 genes were chosen for actual analysis based on their median absolute deviation across the dataset. This was done to reduce background noise in the dataset and to only use genes with high variation across subjects. Consensus clustering separated AML subjects into 5 groups with varying DDR transcriptomic signatures (Fig. 1A). Kaplan-Meier analysis of the survival profiles of the 5 groups revealed cluster 1 displaying the best prognosis while cluster 5 with the worst (Fig. 1B). This prediction was validated by detecting higher frequency of parameters associated with poor prognosis (treatment-refractory cases, prior-MDS, prior-MPN and TP53 mutation) in cluster 5 when compared to cluster 1 (Supplementary Fig. S1). Except consensus sex (more males in cluster 5 vs cluster 1) no other distribution demographics (race, ethnicity, and age) were different in cluster 5 compared to cluster 1 (Supplementary Fig. S2).

The top 10 DDR biomarker genes with the highest expression in each cluster are indicated in Fig. 1C. Individual gene analysis revealed 4 genes in cluster 1 and cluster 5 which enhanced expression correlated with good (AIM2, SATB2, TYMP and TLR4 in cluster 1) and poor (CDC42BPA, LCN2, NF1A and HP in cluster 5) prognosis (Fig. 1D). The most significant cluster marker genes discriminating between good and poor survival were AIM2 (cluster 1 biomarker, high values denoted good prognosis) and CDC42BPA (cluster 5 biomarker, high values denoted bad prognosis).

Absent in melanoma 2 (AIM2) detects DNA in the cytosol and assembles an inflammasome, which activates caspase-1 and pro-inflammatory cytokines leading to pyroptosis, an inflammatory form of cell death [7]. Thus, AIM2 can facilitate pyroptosis in AML cells which accumulate cytosolic DNA during treatment, leading to accelerated elimination of AML cells and favorable prognosis.

CDC42 binding protein kinase alpha (CDC42BPA) also known as the myotonic dystrophy protein kinase-like alpha (MRCKα) is required for TP53-dependent autophagy [8]. Autophagy protects leukemia cells during chemotherapy by providing energy and facilitating proliferation through the supply of essential components such as amino acids and nucleotides. In addition, CDC42BPA has been also implicated in regulation the sensitivity of high-grade serous ovarian carcinoma and glioblastoma cells to chemotherapy and radiotherapy and/or in tumor cell growth [9]. Thus, CDC42BPA might regulate chemotherapy resistance of AML cells and inhibitors of the kinase may have therapeutic application in a cohort of high CDC42BPA expressors [9]. Of note, while TP53 expression did not alter between high and low CDC42BPA expressors, TP53 mutations were found in 19.9% high CDC42BPA expressors, compared to only 3.7% of the low CDC42BPA expressors. Therefore, higher frequency of TP53 mutants among high CDC42BPA expressors might also contribute to worse survival.

Remarkably, combined values of the expression of AIM2 and CDC42BPA are extremely powerful predictor for survival in the training BEAT AML 2.0 database (Fig. 1E). AML patients with high values of AIM2 and low values of CDC42BPA expression displayed the best prognosis, whereas these with low values of AIM2 and high values of CDC42BPA had the worst prognosis. The discovery of biomarker values of AIM2 and CDC42BPA as predictors of survival were validated using TARGET-recurrent AML dataset from 427 patients (Fig. 1E).

AML cells accumulate high numbers of DNA double-strand breaks (DSBs), the most lethal of all DNA lesions resulting from altered metabolism [10, 11] and induced by therapeutic approaches [1]. DSBs are highly deleterious, with a single unrepaired DSB being sufficient to trigger cell death [12]. To repair numerous DSBs AML cells may activate the DSB repair mechanisms involving RAD51-mediated homologous recombination (HR), DNA-PK -mediated non-homologous end-joining (NHEJ) and DNA polymerase theta (Polθ, encoded by POLQ gene)-dependent microhomology-mediated end-joining (TMEJ) [13]

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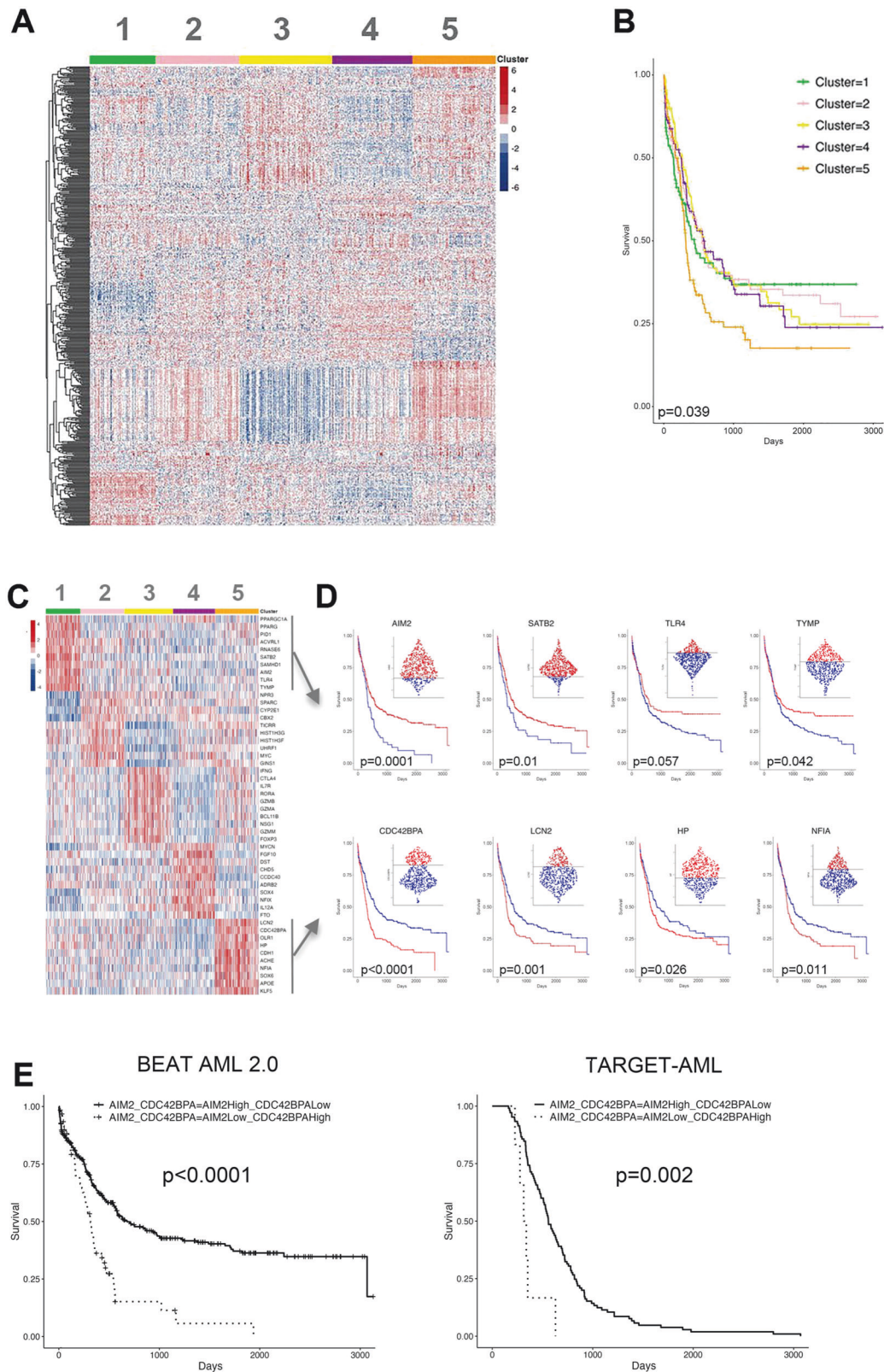
Fig. 1 **DDR genes as biomarkers predicting survival of AML patients. A** Consensus clustering of AML subjects in the BEAT AML 2.0 dataset into 5 groups with varying DDR transcriptomic signatures. **B** Kaplan-Meier plot showing the survival of the 5 groups. **C** Top 10 DDR biomarker genes with the highest expression in each cluster. **D** Kaplan-Meier plots of the difference in survival between subjects with high versus low scaled and normalized values of the DDR biomarker genes for clusters 1 and 5. High versus low cut points were determined via Thiele and Hirschfield's method to find an optimal outcome-based cut point, survival. **E** The combined values of AIM2 and CDC42BPA are a powerful biomarker for survival in BEAT AML 2.0 and TARGET-recurrent AML databases.
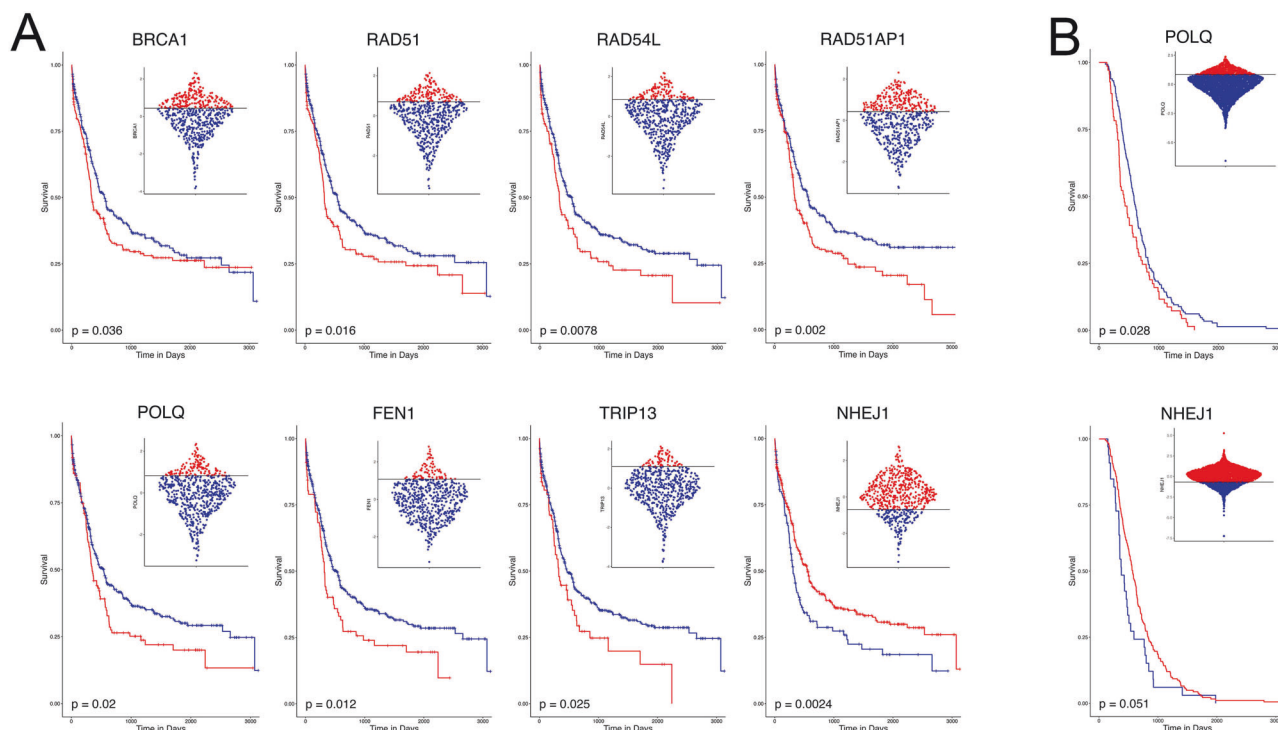
Fig. 2 Expression of DSB repair genes predicts survival of AML. A, B Kaplan-Meier plots showing the difference in survival between subjects with high versus low scaled and normalized values of the DSB repair genes in the BEAT AML 2.0 (**A**) and TARGET-recurrent AML (**B**) datasets. High versus low cut points were determined via Thiele and Hirschfield's method to find an optimal outcome-based cut point, survival.

Therefore, we also tested if expression levels of DSB repair genes had a survival prognostication value.

Analysis of training BEAT AML 2.0 database revealed that upregulation of several genes involved in RAD51-mediated HR (*BRCA1, RAD51, RAD54L* and *RAD51AP1*) and Polθ-dependent TMEJ (*POLQ, FEN1* and *TRIP13*) were associated with worse prognosis (Fig. 2A). Conversely, patients with elevated expression of *NHEJ1* gene (non-homologous end-joining factor 1), a member of NHEJ displayed better prognosis. Remarkably, the biomarker prognosticator values of *POLQ* and *NHEJ1* gene expression were validated in TARGET-recurrent AML dataset (Fig. 2B).

In conclusion, using DDR genes as probes we identified novel biomarkers for AML prognostication: co-expression of *AIM2* and *CDC42BPA* genes and sole expression of *POLQ*. Overexpression of Polθ confers cellular resistance to various genotoxic cancer therapies (ionizing radiation, genotoxic chemotherapy drugs) [14]. The discovery that *POLQ* is a key prognostic biomarker in AML may have a therapeutic value because cancer cells, including AML cells overexpressing Polθ protein are hypersensitive to Polθ inhibitors which are currently in clinical trials against solid tumors [10, 15].

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AUTHOR CONTRIBUTIONS

AK provided bioinformatic analysis, and revised and approved the final version of the manuscript. TS conceived the idea, supervised the project, and wrote the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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